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It's Time to Bring Dendritic Cell Therapy to Type 1 Diabetes

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Type 1 diabetes (T1D) is an autoimmune disease that results from a deficient induction or maintenance of tolerance to islet β -cell antigens, allowing the eventual T-cell-mediated destruction of insulin-producing β -cells within the pancreatic islets (1). Under homeostatic conditions, immune tolerance is established by various subsets of antigen-presenting cells (APCs) with tolerance-inducing/maintaining (tolerogenic) functions and reinforced by other cells with suppressor and immunomodulatory properties. T-cell tolerance manifests itself through elimination (deletion), inactivation (anergy), or suppression of self-reactive T cells (Fig. 1A). These functions may be performed by a variety of tolerogenic APCs (Table 1), some of which have the ability to induce/boost regulatory T cells (Tregs) and/or B cells (Bregs). Genetic and environmental factors that vary among different individuals contribute to the development of T1D, in part by impacting mechanisms of tolerance (Fig. 1B). Therefore, a major goal for the prevention and/or reversal of T1D is to restore effective and durable tolerance to either prevent further destruction of remaining islet β -cells, help islet β -cell regeneration, or protect islet transplants and obviate the use of nonspecific immunosuppressive drugs. The approach reviewed here consists of developing a personalized therapy using the patient's own cells manipulated to perform as tolerogenic APCs (Fig. 1C). In this review, we will explain why dendritic cells (DCs) are the tolerogenic cells of choice to fulfill the goal of restoring immune tolerance in T1D as part of a potentially powerful and safe cellular immunotherapy, the next generation of

therapies (2). We will present the clinical considerations and challenges ahead and the plans to address them, taking into account lessons learned from animal models such as the NOD mouse.

NATURAL TOLEROGENIC APCs

Broadly speaking, tolerogenic APCs use three concomitant or sequential signals to induce tolerance in T cells: 1) an antigenic signal via engagement of the T cell receptor (TCR) by major histocompatibility complex (MHC) molecules bearing peptides from self-antigens; 2) absence of, or impaired, costimulatory confirmatory signals to T cells; and/or 3) tolerogenic signals consisting of inhibitory cell-surface ligands and/or suppressive cytokines, the nature of which varies from one tolerogenic APC population to another (Table 1). Tolerogenic cells of the hematopoietic lineage comprise several DC subsets typically in their steady-state immature stage (3), regulatory macrophages (4) and myeloid-derived suppressive cells (5), the latter being immature precursors of DCs, macrophages, and granulocytes. DCs and macrophages are considered professional APCs; they can acquire antigens in their environment and process these into peptides that are then presented effectively to T cells. In the presence of proinflammatory signals and/or pathogen-derived products, DCs and macrophages mature and convert from a tolerogenic to an immunogenic phenotype by upregulation of MHC and costimulatory molecules and the secretion of proinflammatory cytokines, thereby eliciting a potent immune response. Thus, when

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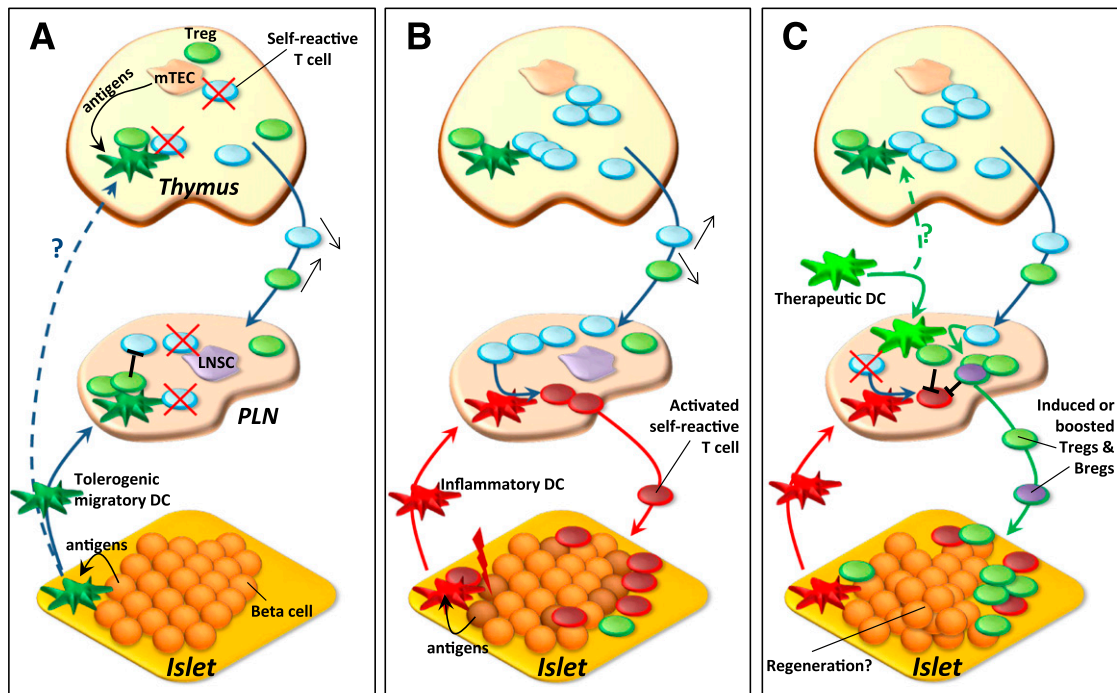


Figure 1—Tolerance to β -cell antigens: induction, loss, and restoration. **A:** Tolerance to β -cell antigens is induced and maintained by tolerogenic cells comprised of migratory DCs transporting antigens from peripheral to lymphoid tissues and mTECs and lymph node stromal cells (LNSCs) ectopically expressing β -cell antigens. This results in the elimination of self-reactive T cells or their conversion into Tregs, which can suppress other self-reactive T cells. **B:** Breakdown of tolerance due to genetic susceptibility and environmental and immunopathological triggers in T1D affect tolerogenic cells at multiple levels: defective expression and tolerogenic presentation of β -cell antigens in lymphoid tissues (leading to increased self-reactive effector vs. Treg relative frequency); islet inflammation leading to immunogenic, rather than tolerogenic, DCs presenting β -cell antigens in the PLNs; and reduced function of tolerogenic cells and regulatory cells. Altogether, these events lead to unopposed activation of β -cell antigen-reactive T cells in the PLNs. **C:** Delivery of engineered therapeutic DCs, particularly those able to target lymphoid sites of tolerance induction, can take over the failing tolerogenic cells and stimulate existing or de novo regulatory cell populations (Tregs and Bregs). In the PLNs in particular, they are able to oppose the action of immunogenic DCs that stimulate the effector function of self-reactive T cells.

it comes to presentation of self-antigens, the same DCs could be either helpful or harmful depending on their maturation stage, which is itself influenced by their environment.

In contrast, tolerogenic cells of the nonhematopoietic lineage, such as stromal cells, are nonprofessional APCs, unable to elicit a productive immune response due to their lack of costimulatory molecules and inability to acquire exogenous antigens for presentation. Instead, they constitutively express inhibitory molecules and are uniquely capable of ectopically expressing tissue-specific antigens for presentation (6–9). These stromal cells include AIRE⁺ (autoimmune regulator) medullary thymic epithelial cells (mTECs) and, in the lymph nodes, fibroblastic reticular cells and lymphatic endothelial cells (LECs). Although these APCs can delete self-reactive CD8⁺ T cells, their role in tolerizing CD4⁺ T cells or inducing Tregs is dependent upon their level of MHC class II expression, ranging from high in mTECs to absent in LECs (10). Mesenchymal stromal cells also support Treg development, although it is not clear whether they can act as APCs (11). In addition, AIRE⁺ stromal and/or DCs are also present in the lymph nodes and spleen (6,12,13).

Although DCs have been found to express some self-antigens ectopically (14,15), they can also cross-present antigens acquired from stromal cells (16) or other DCs with which they come into contact (17). In addition, because DCs are motile cells, they can pick up antigens in tissues before migrating to present them in the draining lymph nodes or the thymus (18–20). Moreover, DCs inside lymph nodes can acquire antigens derived from tissues and organs that are naturally drained by afferent lymphatics. Although targeting antigens to migratory DCs in vivo leads to better Treg induction compared with lymphoid-resident DCs (21), the migratory pathways and the interactions among DCs, lymphatics, lymphatic drainage, and the complement of antigens provided by the tissues draining into lymphatics into which DCs circulate can obviate the need to provide specific antigens exogenously.

WHY USE DCs AS THERAPEUTIC TOLEROGENTIC CELLS?

There are a number of good reasons to consider DCs for therapeutic use in humans. First, DCs can be generated from the most readily available source: the blood. They

Table 1 — Natural tolerogenic cells versus human monocyte-derived DCs

Subset	Distribution	Phenotype	Antigen expression	Tolerogenic function
Classical DCs (with tolerogenic properties)	Migratory, from tissues to lymph nodes, spleen, and thymus	CD11c ⁺ MHC-II ⁺ BDCA-1 ⁺ CD11b⁺	Acquired in tissues, draining lymph nodes, or stromal cells	T-cell deletion or anergy, Treg induction via IL-10
Plasmacytoid DCs	Migratory, from tissues to lymph nodes, spleen, and thymus	MHC-II ⁺ BDCA-2/4 ⁺ CD123 ⁺ PDCA-1⁺ B220⁺	Acquired in tissues, draining lymph nodes, or stromal cells	T-cell deletion or anergy, Treg induction via IL-10
Myeloid-derived suppressor cells	Migratory, from bone marrow to lymphoid tissues and tumors	CD11b ⁺ CD14 ⁻ CD33 ⁺ Gr-1⁺	Not required	Inhibition of T-cell proliferation via NO, Arg1
Regulatory macrophages	Resident	CD11c ⁺ F4/80 ⁺ Siglec F ⁺	Not required, but may occur	Treg induction via TGF-β and RA
MECs	Resident, thymus	AIRE ⁺ UEA-1 ⁺ MHC-II ⁺ EpCAM ⁺	Ectopic, AIRE dependent	CD4 ⁺ and CD8 ⁺ T-cell deletion, Treg induction
eTACs	Resident?, lymph nodes and spleen	AIRE ⁺ UEA-1 ⁻ MHC-II ⁺ EpCAM ⁺	Ectopic, AIRE dependent	CD8 ⁺ T-cell deletion, CD4 ⁺ T-cell anergy
FRCs	Resident, lymph nodes	CD45 ⁻ CD31 ⁻ PDPN ⁺	Ectopic	CD8 ⁺ T-cell deletion, inhibition of T-cell proliferation via NO
LECs	Resident, lymph nodes	CD45 ⁻ CD31 ⁺ PDPN ⁺	Ectopic, AIRE independent	CD8 ⁺ T-cell deletion via PD-L1
Monocyte-derived DCs	Generated in vitro with GM-CSF and IL-4	CD11c ⁺ MHC-II ⁺ CD209 ⁺	Antigen can be delivered in vitro or acquired in vivo	Varies depending on the modifications applied in vitro

Phenotype: markers expressed in both humans and mice, unless underlined (humans only) or in boldface (mice only). Arg1, arginase 1; eTACs, extrathymic Aire-expressing cells; FRCs, fibroblastic reticular cells; MECs, medullary epithelial cells; NO, nitric oxide; PDPN, podoplanin; RA, retinoic acid.

Table 2—Ex vivo manipulations used to enhance the tolerogenic/immunoregulatory function of DCs

	Products used: IL-10, TGF- β , IFN- γ , GM-CSF, vitamin D3, cyclosporine A, dexamethasone, rapamycin, monophosphoryl lipid A, deoxyspergualin, mycophenolate mofetil, NF- κ B inhibitors, <i>Aspergillus oryzae</i> proteases, curcumin, glycodelin A
Culture medium supplementation	
Gene silencing (antisense oligonucleotides, double-stranded transcriptional decoys)	Products silenced: NF- κ B, RelB, CD40, CD80, CD86
Adenoviral transduction	Products expressed: IL-4, IL-10, TGF- β , galectin-1, CTLA4-Ig, IDO
Lentiviral transduction (and retroviral transduction)	Products expressed: IL-4, IL-10, CTLA4-Ig, SOCS-3, RelB shRNA
DNA transfection	Products expressed: IDO, FasL
mRNA electroporation	Products expressed: IL-4

References for most of these studies can be found in several reviews (23–26). IDO, indoleamine 2,3-dioxygenase; IFN- γ , interferon- γ ; shRNA, short-hairpin RNA; SOCS-3, suppressor of cytokine signaling-3.

can be differentiated from monocytes using the cytokines granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-4 (IL-4) over the course of a 1-week culture. Monocytes represent ~10% of peripheral blood mononucleated cells, and usually, 40,000 to 100,000 DCs can be eventually recovered per milliliter of blood. In the clinics, large numbers of peripheral blood mononucleated cells are now obtained by leukapheresis, meaning that the other components of the blood are returned to the circulation. In contrast, other types of tissue-populating DCs and stromal cells are much more difficult to isolate and propagate. Moreover, stromal cells rely on their low endogenous expression for antigens due to their inability to capture them, expression that is usually lost over time, outside their natural niche in vivo.

Second, tolerogenic DCs are typically migratory in nature (21), and therapeutic DCs of this type are capable of migrating from sites of inoculation to relevant target tissues on their own (22). This propensity can be enhanced by modulating their expression of chemokine receptors and/or integrins. In contrast, it is unclear whether stromal cells, if generated in vitro, would have the ability to migrate to tissues of interest, or even survive in vivo.

Third, DCs are amenable to manipulation in vitro, whether by simply modifying or supplementing the culture conditions or by actively silencing or overexpressing genes. Such manipulations are nonexhaustively outlined in Table 2 and are reviewed in more details elsewhere (23–26). Although it is possible to target existing DCs in vivo for example via DC-specific antibodies (27) or microparticles (28,29), manipulation ex vivo offers a higher level of control and flexibility for altering gene expression and is required when overexpression of particular genes or transgenes is sought. All modifications listed in Table 2 have conferred upon bone marrow-derived DCs (CD11c⁺ CD11b⁺ MHC-II⁺) the ability to delay, prevent, and in some cases reverse disease in NOD mice.

Like human monocyte-derived DCs (CD11c⁺ CD209⁺ HLA-DR⁺), these DCs are also generated using GM-CSF and IL-4. These kinds of preclinical studies should always use unmanipulated DCs for comparison, as the latter have some therapeutic value on their own. Manipulation of DCs may also overcome possible intrinsic defects in their function that may have played a role in the disease progression in the first place.

Fourth, DCs regulate the activity, persistence, and half-life of other regulatory cells, including Foxp3⁺ CD4⁺ Tregs (30), CD8⁺ Tregs (31), and Bregs (32). This attribute increases the probability that multiple layers of tolerance can be built and maintained where antigen specificity is a dynamic process over the evolution of late-stage autoimmunity or at clinical onset.

One of the most desired outcomes sought from the engineering of DCs ex vivo or in vivo is to increase their ability to induce or boost regulatory cells for long-lasting immunoregulation. Aiming to achieve deletion or anergy of self-reactive T cells therapeutically may not have as long-lasting an effect since the pool of self-reactive T cells is replenished over time, thereby requiring continuous tolerogenic presentation of relevant antigens. One approach, reviewed elsewhere, consists of isolating and expanding, or engineering antigen-specific Tregs ex vivo (33). This strategy has its own benefits and challenges; however, we would argue that DCs have the potential to induce different types of regulatory cells (adaptive Tregs such as Tr1/Th3, Bregs, and Th2) and/or bolster the function of pre-existing thymic-derived Tregs (34,35). These properties are highly relevant not only for T1D but for treating other autoimmune diseases and for preventing transplantation rejection. The efficacy of tolerogenic DCs correlates greatly with the appearance and/or increased function of these different regulatory cells.

Antigen-specific tolerance has usually been observed in models involving the adoptive transfer of TCR-transgenic T cells in which antigen-specific deletion, anergy, or Treg

induction can be easily evaluated. An increase in the frequency or function of Foxp3⁺ Tregs may follow the administration of therapeutic DCs, but it is difficult to assess whether these cells recognize islet antigens and could initiate and propagate reverse epitope spreading by linked suppression. Moreover, the stability of Tregs is crucial for the long-term maintenance of tolerance, an aspect that has been greatly emphasized recently. Demethylation of two regions in the Foxp3 promoter region enables stability, whereas partial demethylation may render the Tregs susceptible to conversion into effector T cells under certain conditions (36). Thus, when Tregs are induced by DC therapy, stability is another parameter to evaluate in addition to suppressive function. Less typically, DC-based therapy has been associated with other types of protective cells, including induction of IL-10-producing suppressive B cells (suggestive of Bregs) (32,34) and immune deviation of effector T cells toward a Th2 phenotype (counteracting the more pathogenic Th1 phenotype) (35,37–39). These cells can be significant suppressors of autoimmune processes, especially if present in critical sites such as the pancreatic lymph nodes (PLNs) or the islets. Although therapeutic DCs may eliminate or anergize some diabetogenic T cells during the short time they persist *in vivo*, the suppressive milieu they create or the regulatory populations they induce are likely to be the key factors in perpetuating long-term tolerance induction.

IS DC THERAPY EFFECTIVE AND SAFE?

Efficacy and safety are two major concerns for the translation of successful animal studies into the clinic. Efficacy remains the biggest challenge, given that many successful therapies in NOD mice, cellular or other, led to disappointing results when tested in humans. The possible reasons are numerous, but the heterogeneity of human patients (both genetic and the environment in which they live) is a major factor when compared with a genetically homogenous strain of mice bred in a controlled environment. Combination therapies are increasingly considered as a way to tackle the heterogeneity of human patients by impacting multiple biological pathways, hoping to block at least one that might be defective. DC-based therapy has unique benefits in that respect, which will be discussed later. Before efficacy of various types of DC manipulations can be determined in humans, safety must be established. To this end, a phase I clinical trial with 10 subjects has already been conducted in T1D patients and demonstrated the safety of DCs, with or without manipulation *in vitro* (34). This safety aspect is also supported by a large number of clinical trials using modified DCs to treat cancer and certain chronic infections, including the approval of Provenge as a DC vaccine for prostate cancer (40–42). Although these therapies have exploited the immunogenic rather than tolerogenic side of DC therapy, Tregs were also induced (43). All the clinical studies conducted

so far have demonstrated that DCs do not result in any serious adverse effects commonly seen with most of the drugs and biologics used to treat these diseases. Given the safety profile of DCs in past and ongoing clinical trials, as well as the extensive literature demonstrating the clinical benefits of various DC manipulations, including the ability to control the presentation of relevant antigen epitopes and the expression of homing molecules for better cell and tissue targeting, and the ability to combine these manipulations, we consider that this is the time to address the following question: “Why not consider the use of DC-based therapy to treat T1D?”

PRODUCING AND MANIPULATING CLINICAL-GRADE DCs

DC-based treatment of human diseases has been done predominantly with monocyte-derived DCs. In the past decades, significant improvements have been made on the yield and quality of the DCs generated from leukapheresis and on the automation of the procedure, all under strict good manufacturing practice (GMP) standards (41). Such standards are in place to ensure that the infused cell mixture and the products used to enhance their tolerogenic properties are free of contaminants of any sort, and that DCs are stably tolerogenic. In these individualized cell therapies, each “drug” (autologous DCs or Tregs under current scenarios) is unique, and as a result, the cost is significantly higher than more traditional drug treatments. However, if a single course of treatment, possibly with occasional “boosting,” can achieve the goal of durable tolerance restoration, prevention of disease progression, insulin independence, and/or prevention of long-term complications, the approach would be highly cost-effective. Improvements in DC cryopreservation techniques also allow multiple treatments to be performed from a single preparation, thereby reducing subsequent production costs.

As previously mentioned, DCs must be treated or manipulated *ex vivo* for them to acquire or maintain tolerogenic properties, including the addition of agents during DC preparation to influence their phenotype, the delivery of oligonucleotides aimed at silencing genes involved in DC maturation (effectively locking DCs in an immature/tolerogenic state), and/or the transfection or transduction of the DCs to overexpress tolerogenic ligands or cytokines (Table 2). The “material” used for DC manipulation must be prepared under GMP standards. For gene silencing or gene overexpression in human DCs for the purpose of immunotherapy, the methods of choice have been antisense DNA oligonucleotides and mRNA electroporation, respectively. Viral vectors, although widely used in preclinical studies to more stably modify DCs, have not been applied in human clinical trials due to safety concerns over potential risks of malignancy (disruption of a tumor suppressor gene upon chromosomal integration) and immunogenicity (expression of viral antigens and/or maturation of DCs

through activation of Toll-like receptors and other pattern-recognition receptors). Furthermore, viral vectors are limited in the number of products they can deliver to cells, and the transduction efficiency and vector expression characteristics vary widely among viral systems. Long-term expression of immunomodulatory genes may be undesirable beyond a point when disease-specific immune tolerance has been restored. Although DCs appear to survive only 1–2 weeks *in vivo* after injection (22), the gene expression itself can be rendered more transient by transfection of nonintegrating DNA or by electroporation with mRNA. Such transient expression of regulatory genes may be sufficient when the goal is to induce regulatory cells that themselves are long-lived, or an immunomodulatory milieu that will perpetuate the control of the disease long after the DCs are gone (35). An additional advantage of DNA oligonucleotides and mRNA is the ability to use mixtures to silence or overexpress multiple genes (34,44).

Monocyte-derived DCs are now part of a number of clinical trials for autologous therapeutic vaccination of various types of cancers (40). The first treatment of this kind to be FDA approved (Provenge) is now available for the treatment of prostate cancer, having demonstrated a significant life-prolonging effect (42). As DC-based therapy is applied to more diseases, such as T1D (34) and rheumatoid arthritis (45), the cost will be expected to go down as methods of production and manipulation become more standardized and automated. Furthermore, the number of GMP facilities in the U.S. is expanding as cell therapies with stem cells, T cells, and DCs are becoming more widespread.

Given the limited number of GMP facilities certified for clinical-grade DC therapy products among academic institutes, hospitals, and for-profit biotechnology companies, one concern lies in the establishment of standard operating procedures to receive leukapheresis packets, process the DCs, and deliver them to distant sites. Fortunately, this work has already been conducted in the realm of cancer immunotherapy (46), and SOPs can be modeled on this process for tolerogenic DCs. Standardization of assays for potency can be readily adapted among laboratories and centers participating in tolerogenic DC clinical trials and can include flow cytometric measurements and cytokine responses to ensure that the delivered thawed cell product is viable and phenotypically identical to the freshly prepared DCs.

TARGETING THE RELEVANT T CELLS: IS ANTIGEN PROVISION TO THE TOLEROGENIC DC REQUIRED?

Highly relevant to tolerance induction is the question regarding the requirement for antigen-specific signals. Although immunoregulatory products delivered locally have been demonstrated to suppress autoimmune responses and reduce inflammation without provision of exogenous antigens (35,38), some tolerogenic signals require concomitant TCR cross-linking for the T cells to

become properly tolerized or acquire a regulatory phenotype. Provision of antigens to DCs before administration is one way to assure antigen specificity, something made possible by the knowledge of many T1D-relevant targeted epitopes involved in mice and humans (47). Thus, one can provide proteins or peptides to the DCs toward the end of their culture *in vitro* such that they present major epitopes *in vivo* to specifically target diabetogenic T cells, conceivably including major drivers of the disease. Some antigen-specific Tregs may already exist, waiting to be boosted by appropriate signals provided by tolerogenic DCs. Other regulatory populations may or may not require antigen. For example, Bregs are induced after DC treatment (without antigen), at least in part through the action of retinoic acid (32,34). Some of them produce IL-10, although others apparently do not require IL-10 for suppression (32). Their antigen specificity remains unclear.

However, T1D, like many other autoimmune diseases, is characterized by epitope spreading, meaning that the number of targeted epitopes increases as the disease progresses. Although covering all targeted antigens and epitopes may not be possible, the concept of linked suppression may be exploited to also “spread suppression” from one epitope to another using tolerogenic DCs (48). The second possibility is to let DCs acquire antigens themselves from their environment. As previously mentioned, DCs have several ways to do so. According to preclinical studies in NOD mice, although DCs under certain conditions provide better protection with antigen provision (49), in many other studies, they do not require antigen supplementation to mediate immunoregulatory functions (35,38,50,51). It is possible that the suppression by DCs that were not given antigens has a non-antigen-specific component (52), as well as an antigen-specific component, through the processing and presentation of self-antigens acquired *in vivo* from relevant tissues. The PLNs, which directly drain the pancreas, may represent a reservoir of islet antigens, either released by injured islet cells, ectopically expressed by resident cells (14,15,53), or possibly cross-presented from DCs activated in the islets. The need to counteract the immunogenic function of these activated DCs makes the PLNs a prime target for the action of tolerogenic DCs.

DEALING WITH TWO FACETS OF DC MATURITY

The main disadvantage of DCs compared with stromal cells is that the same cell can be tolerogenic or immunogenic depending on its environment. Immunogenicity is often associated with DC maturity, but again, the environment may dictate otherwise. For example, migratory DCs that enter the thymus undergo maturation, yet deliver tolerogenic signals (54), which may also reflect different requirements for tolerance induction in thymocytes as opposed to mature peripheral T cells. DC maturation has two major components, the first is the

upregulation of MHC and costimulatory molecules and the second is the production of proinflammatory cytokines (IL-1, IL-6, and tumor necrosis factor) and T-helper cell-skewing cytokines such as IL-12. This distinction is important as semimature DCs, that express higher surface levels of costimulatory molecules but do not secrete proinflammatory cytokines, also have tolerogenic properties (55). Such semimature DCs, generated with GM-CSF and IL-4, were more protective in NOD mice than completely immature DCs obtained with GM-CSF only (50). The protection conferred by these semimature DCs may reflect the activation of protective Th2 cells more than induction of Tregs (37,55), possibly suggesting that DCs in different states of maturity can contribute to tolerance induction through different mechanisms. Semimature human DCs can be obtained with the addition of tumor necrosis factor- α to the usual cytokine cocktail, whereas full maturation can be achieved with the addition of microbial products such as LPS (55).

In cancer vaccine immunotherapy, mature DCs are desirable for optimal immunogenicity (40) and reversion to an immature state has never been shown, as maturation represents a terminal differentiation. However, full maturation should be avoided in the case of autoimmunity to prevent disease exacerbation. DCs that are functionally tolerogenic *in vitro* should remain so *in vivo* after administration. However, cell plasticity (phenotypic and functional instability) is an issue that concerns all cell therapies. Thus, it is crucial to enforce a stable and persistent state of immaturity or semimaturity that cannot be undone by environmental signals *in vivo* for the duration of life of the administered DCs. Whether the DCs are provided antigens or acquire them in their environment *in vivo*, they may be exposed to inflammatory conditions, which could provoke full maturation, thereby carrying the risk of activating rather than tolerizing antigen-specific T cells and thus aggravating the disease. Approaches aimed at locking the DCs in an immature state without the ability to achieve full maturation or making the DCs deliver tolerogenic signals so potent that they cannot be affected by inflammation are important safety aspects to be considered using this type of therapy. For example, the first safety trial performed in patients with T1D used DCs generated *ex vivo* in the presence of antisense oligonucleotides that silenced CD40, CD80, and CD86 (34). This method has proven efficient in preventing the maturation of mouse DCs (28), as did glucocorticoids (56), and other methods targeting the nuclear factor- κ B (NF- κ B) pathway (57,58), which can also interfere with the signals leading to the production of cytokines such as IL-12. When diabetogenic antigens are provided, the testing of DCs should include a demonstration that gene silencing of immunogenic factors or overexpression of tolerogenic products outlasts the presentation of these antigens.

In sum, an important part of the product quality control will be to ensure that manipulations performed *in vitro* intended specifically to control the phenotype of the DCs are both stable throughout the remaining lifetime of the cell and resistant to exogenous insults. Other manipulations intended to boost the tolerogenic function of the DCs may be more transient.

TARGETING RELEVANT TISSUES WITH DCs

Imaging and biodistribution studies in animals have revealed important aspects of the natural homing of therapeutic DCs. Relevant to T1D, substantial accumulation of *ex vivo*-expanded DCs has been observed in the PLNs of mice after their intravenous or intraperitoneal injection (Fig. 2) (22,38). DCs injected subcutaneously in appropriate areas of the abdomen in mice and monkeys can also home to the PLNs (N.G. and M.T., unpublished observations). This specific homing has important implications: therapeutic DCs have better access to relevant antigens in this tissue, they are on the frontline to oppose the action of islet-derived immunogenic DCs, and the amount of tolerogenic “product” required to block disease is much less than the amount of the product required to be delivered systemically, a form of targeted immunotherapy. In addition to tolerogenic products, lymph node-homing signals, such as CCR7 and L-selectin, could be overexpressed to enhance the accumulation of more immature DCs (59). Enhanced tissue targeting is a good way to improve both the efficiency and safety of therapy.

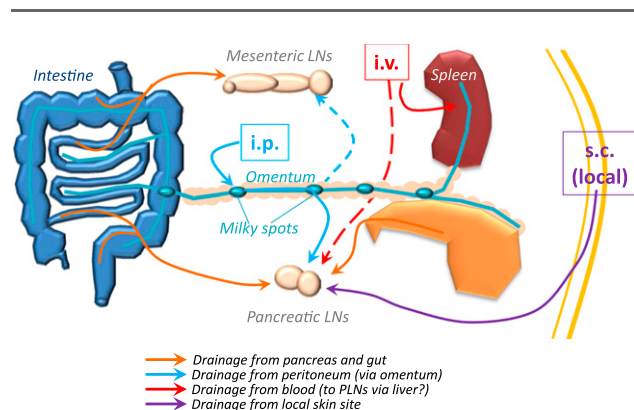


Figure 2—The PLNs, Grand Central Station for DCs? Mouse studies have unveiled a remarkably central positioning of the PLNs in immune surveillance. They drain not only the pancreas very efficiently but also the gut and the peritoneum (22,67). After intravenous (i.v.) and intraperitoneal (i.p.) injection, DCs accumulate preferentially in the PLNs, although the mechanism of the former route is not understood (22). In addition, subcutaneous (s.c.) inoculation in an abdominal site also allows targeting of DCs to the PLNs (N.G. and M.T., unpublished results). Drainage patterns in humans are not as well defined but are likely to be more complex. Combining specific routes to achieve proper drainage together with the use of chemokine receptors and integrins to enhance homing will permit more targeted and efficient tolerance-inducing therapies. LN, lymph nodes.

Imaging of radiolabeled DCs in humans has also demonstrated homing of DCs to vascularized organs (lungs, liver, and spleen) after intravenous injection or to local lymph nodes after subcutaneous injection (see discussion in reference 22 for references). Fine imaging of DC homing to deep lymph nodes like the PLNs is challenging due to sensitivity issues. The homing pattern associated with different routes of inoculation in mice remains to be tested in humans. The next step in that respect would be to do more extensive studies in non-human primates or to analyze PLNs collected by surgery or laparoscopy from patients after intravenous, intraperitoneal, or subcutaneous inoculation of DCs in current cancer therapy models. Although not ordinarily performed in humans, and more difficult to carry out, intraperitoneal, omental, or paragastric intranodal injection could be considered as it appears to be the most efficient and most specific route for PLN targeting, at least in mice. Systemic delivery by intravenous infusion allows the targeting of other secondary lymphoid tissues, such as the spleen, that may also be important for tolerance induction (Fig. 2). Thus, it may be advantageous to study the combination of multiple routes for any one treatment to extend the reach of the therapeutic DCs.

DECIDING ON THE TIME OF INTERVENTION, DOSE, AND NUMBER OF INJECTIONS

In the NOD model, the disease is predictable based on the age of mice and the disease incidence in the colony. Autoantibodies in NOD mice are usually detected around 8–10 weeks, knowing that initiation probably takes place around 4 weeks and the earliest onset of disease around 12 weeks. We learned from these mouse studies that the time of intervention is an important consideration for efficacy. For example, unmanipulated DCs can prevent disease onset in NOD mice when administered at a young age (4–8 weeks) but cannot when administered shortly before the onset of disease (at 10–12 weeks), whereas the expression of IL-4 by the same DCs conferred protection in these later stages of disease (35,38,39,60). In preclinical studies in rodent models, there are several successful therapies that reverse disease or prevent onset of hyperglycemia at a late stage compared with many that show efficacy when given to young animals, but those approaches that work in later stages of NOD disease show more promising potential for translation to human patients (61).

The same may apply to humans, who can be treated shortly after or before onset of hyperglycemia in individuals with a high probability of imminent onset. In T1D patients with recent onset, it may be possible, yet challenging, to rescue remaining viable β -cells or allow β -cell regeneration to provide sufficient insulin secretion, but this may require concomitant relief from the stress of inflammation. Individuals at very high risk of developing hyperglycemia, based on family history, HLA genotype, and the presence of autoantibodies, are good candidates

for prevention by DC-based immunotherapy (62). We believe that DC therapy would be suitable for prevention in high-risk subjects, after adequate safety assessment based on the issues discussed above, because autologous DCs are safe and well tolerated in humans, and because current DC formulations in preclinical studies are more effective in disease prevention than reversal. Our ability to detect autoimmunity and predict the level of risk for T1D has greatly improved. Although it is not yet possible to determine with absolute and unequivocal certainty when islet inflammation begins, its existence may be identified by islet-specific autoantibodies as well as biomarkers, including circulating demethylated insulin DNA (63) or miR-375 (64). In addition, other recent data suggest that loss of glucose sensitivity upon oral glucose tolerance testing may indicate a relatively imminent onset of hyperglycemia in T1D (65).

As for any vaccine, the dose and the number of treatments are extrapolated from preclinical studies, in which they are themselves empirically determined. This leads to extremely high variability of the conditions selected in preclinical studies (number of cells injected, time and number of treatments, etc.) and makes comparisons difficult. In NOD mice, DC therapy typically consists of $0.5\text{--}5 \times 10^6$ cells administered either once or by several injections at weekly intervals, and in the first human T1D trial, DCs were injected four times, once every other week (34). Prior to this safety trial, a small study conducted on two patients had first suggested the ability of immature DCs to inhibit T-cell responses in an antigen-specific manner, using as few as 2×10^6 DCs injected subcutaneously (66). The field of cancer immunotherapy using DCs is more advanced, with many clinical trials completed or ongoing (see reference 40 for examples). In these studies, DCs are inoculated one to five times, at intervals ranging from 2 to 8 weeks and at a dose of $1\text{--}50 \times 10^6$ cells per injection. The cells are administered by subcutaneous or intradermal injection (at sites proximal to lymph nodes), intranodal injection, or intravenous infusion (particularly for a large number of cells). Absence of major adverse effects was noted even with the administration of large numbers of cells. However, these numbers are not up to the scale (number of cells administered per body weight) of those typically used in animals. Thus, a compromise needs to be found to achieve the desired therapeutic outcome with an acceptable number of cells. Again, improving tissue targeting will no doubt help reduce the number of cells, because ultimately, it is the number of DCs homing to the relevant sites that matters, not the total number of DCs injected.

Since a single administration of tolerogenic DCs has generally proven effective in NOD mice, prime-boost strategies have rarely been tested. In several instances, a “booster” treatment failed to provide therapeutic benefit (R.J.C. and C.G.F., unpublished observations), and in others, more than one injection of immature DCs was needed for a more sustained Treg induction (M.J.C.-S.,

unpublished observations) in these mice. Once safety has been established for a particular DC preparation and type of manipulation, multiple administrations may be required to potentially increase the success rate of therapy. This can be done by the use of cryopreserved vials of DCs, ready for inoculation.

ENHANCING THE TOLEROGENIC FUNCTION OF DCs WITH COMBINATION THERAPIES

Three types of combination therapies can be performed with DCs. In the first type, the combination is within the DCs, wherein a number of tolerogenic products may be overexpressed in combination, in order to engage multiple biological pathways in the target T cells. These kinds of combinations can easily be achieved using mRNA electroporation (44). These engagements may replace the action of biologics and, because they are all codelivered by the same DC, ensure the integration of all signals by the target T cells.

The second type of combination therapy could involve a mixture of different populations of DCs, each with their own modifications. Thus, each population can be specialized in a particular task, and each may have its own homing enhancement. Or, as previously suggested, the same DCs can be inoculated through multiple routes, for example intravenous and intraperitoneal, in order to achieve potentially maximal accumulation in the spleen and PLNs.

The third type of combination therapy would ally DCs with a drug or a biologic. For example, concomitant anti-inflammatory therapy or short-term depletion of effector T cells would be beneficial to not only enhance the tolerogenic function but also reinforce the stability of the phenotype (resistance to full maturation) of tolerogenic DCs. Although the DCs can be made to produce anti-inflammatory cytokines (IL-10 and transforming growth factor [TGF]- β) themselves, anti-inflammatory or T-cell-depleting drugs could be given to the patient ahead of the DC inoculation to "prepare the field." Perhaps in a more distant future, DCs could conceivably be used to boost the function of "memory" Tregs, by themselves or after Treg therapy, as part of a reverse vaccination scheme of maintenance rather than induction of tolerance.

CONCLUSIONS

Despite many overlapping features between the mouse and human T1D (1), a substantial genetic heterogeneity as well as differential environmental exposures exists among humans developing T1D, and the sum of these genetic and environmental factors determines disease progression and the onset of hyperglycemia, whereas NOD mice are genetically uniform animals and are housed in environmentally similar conditions. It is therefore easier to prevent T1D progression in NOD mice using treatments, including DC-based approaches, at disease stages that are well defined. We believe that it is also possible to prevent or treat T1D in humans; we now

have phase I safety data in T1D subjects treated with engineered DCs (34), the sites of tolerance induction are better understood, the different cells with tolerogenic potential are better known, and the tolerogenic pathways and molecules involved are better characterized. With this knowledge, we should be able to engineer therapeutic DCs with all the "tools" they need to block T1D progression, tools to allow better migration to relevant sites of tolerance induction and to more efficiently create a protective milieu and/or stimulate regulatory cells. With new ways to more safely manipulate human monocyte-derived DCs *ex vivo*, we can have them perform more complex functions, possibly engaging self-reactive T cells through multiple pathways for better and more durable "reprogramming," thereby conquering the heterogeneity of deficiencies to be overcome in humans.

The treatment of autoimmune diseases using DCs is lagging behind that of cancer, as cell-based therapy tends to be regarded as a weapon of last resort, and more readily used in cancer given the more imminently life-threatening aspect of cancer compared with autoimmunity. Yet we see a strong rationale for such cell-based approach given not only its safety record but also its flexibility and potentially powerful tolerogenic effect(s). Cell-based therapies afford many other benefits that conventional drugs do not provide, including specific migration for targeted delivery of therapy and ability to respond to their environment and to simultaneously perform multiple tasks if engineered to do so (2). Eventually, these personalized treatments can be adjusted and optimized for the patient as we become better equipped to evaluate their genetic defects and the antigens and environmental factors driving their autoimmune response. The safe outcome of the initial safety trial (34) lays the foundation upon which we can confidently begin to design and clinically implement innovative methods to generate and use tolerogenic DCs. It is our conviction that the age of personalized cell therapy in the form of tolerogenic DCs has arrived and that T1D could be the first autoimmune disorder to be successfully treated. Concerted and collaborative approaches using such tolerogenic DCs can accelerate the identification of the most efficacious embodiment of therapy: tolerogenic product delivered, dose, as well as frequency and route of administration that will reverse the new onset of disease and/or prevent the conversion of T1D autoimmunity into clinical hyperglycemia.

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References

- Todd JA. Etiology of type 1 diabetes. *Immunity* 2010;32:457–467
- Fischbach MA, Bluestone JA, Lim WA. Cell-based therapeutics: the next pillar of medicine. *Sci Transl Med* 2013;5:179ps7
- Manicassamy S, Pulendran B. Dendritic cell control of tolerogenic responses. *Immunol Rev* 2011;241:206–227
- Soroosh P, Doherty TA, Duan W, et al. Lung-resident tissue macrophages generate Foxp3+ regulatory T cells and promote airway tolerance. *J Exp Med* 2013;210:775–788
- Yin B, Ma G, Yen CY, et al. Myeloid-derived suppressor cells prevent type 1 diabetes in murine models. *J Immunol* 2010;185:5828–5834
- Gardner JM, Devoss JJ, Friedman RS, et al. Deletional tolerance mediated by extrathymic Aire-expressing cells. *Science* 2008;321:843–847
- Fletcher AL, Lukacs-Kornek V, Reynoso ED, et al. Lymph node fibroblastic reticular cells directly present peripheral tissue antigen under steady-state and inflammatory conditions. *J Exp Med* 2010;207:689–697
- Cohen JN, Guidi CJ, Tewalt EF, et al. Lymph node-resident lymphatic endothelial cells mediate peripheral tolerance via Aire-independent direct antigen presentation. *J Exp Med* 2010;207:681–688
- Yip L, Creusot RJ, Pager CT, Sarnow P, Fathman CG. Reduced DEAF1 function during type 1 diabetes inhibits translation in lymph node stromal cells by suppressing Eif4g3. *J Mol Cell Biol* 2013;5:99–110
- Cowan JE, Parnell SM, Nakamura K, et al. The thymic medulla is required for Foxp3+ regulatory but not conventional CD4+ thymocyte development. *J Exp Med* 2013;210:675–681
- Burr SP, Dazzi F, Garden OA. Mesenchymal stromal cells and regulatory T cells: the yin and yang of peripheral tolerance? *Immunol Cell Biol* 2013;91:12–18
- Grupillo M, Gualtierotti G, He J, et al. Essential roles of insulin expression in Aire+ tolerogenic dendritic cells in maintaining peripheral self-tolerance of islet β -cells. *Cell Immunol* 2012;273:115–123
- Poliani PL, Kisand K, Marrella V, et al. Human peripheral lymphoid tissues contain autoimmune regulator-expressing dendritic cells. *Am J Pathol* 2010;176:1104–1112
- Garcia CA, Prabakar KR, Diez J, et al. Dendritic cells in human thymus and periphery display a proinsulin epitope in a transcription-dependent, capture-independent fashion. *J Immunol* 2005;175:2111–2122
- Pugliese A, Brown D, Garza D, et al. Self-antigen-presenting cells expressing diabetes-associated autoantigens exist in both thymus and peripheral lymphoid organs. *J Clin Invest* 2001;107:555–564
- Joffre OP, Segura E, Savina A, Amigorena S. Cross-presentation by dendritic cells. *Nat Rev Immunol* 2012;12:557–569
- Carbone FR, Belz GT, Heath WR. Transfer of antigen between migrating and lymph node-resident DCs in peripheral T-cell tolerance and immunity. *Trends Immunol* 2004;25:655–658
- Proietto AI, van Dommelen S, Zhou P, et al. Dendritic cells in the thymus contribute to T-regulatory cell induction. *Proc Natl Acad Sci USA* 2008;105:19869–19874
- Hadeiba H, Sato T, Habtezion A, Oderup C, Pan J, Butcher EC. CCR9 expression defines tolerogenic plasmacytoid dendritic cells able to suppress acute graft-versus-host disease. *Nat Immunol* 2008;9:1253–1260
- Kyewski BA, Fathman CG, Rouse RV. Intrathymic presentation of circulating non-MHC antigens by medullary dendritic cells. An antigen-dependent microenvironment for T cell differentiation. *J Exp Med* 1986;163:231–246
- Idoyaga J, Fiorese C, Zbytniuk L, et al. Specialized role of migratory dendritic cells in peripheral tolerance induction. *J Clin Invest* 2013;123:844–854
- Creusot RJ, Yaghoubi SS, Chang P, et al. Lymphoid-tissue-specific homing of bone-marrow-derived dendritic cells. *Blood* 2009;113:6638–6647
- Nikolic T, Roep BO. Regulatory multitasking of tolerogenic dendritic cells – lessons taken from vitamin d3-treated tolerogenic dendritic cells. *Front Immunol* 2013;4:113
- Phillips B, Trucco M, Giannoukakis N. Current state of type 1 diabetes immunotherapy: incremental advances, huge leaps, or more of the same? *Clin Dev Immunol* 2011;2011:432016
- Giannoukakis N, Trucco M. Dendritic cell therapy for type 1 diabetes suppression. *Immunotherapy* 2012;4:1063–1074
- Humbert JM, Halary F. Viral and non-viral methods to genetically modify dendritic cells. *Curr Gene Ther* 2012;12:127–136
- Mukherjee G, Dilorenzo TP. The immunotherapeutic potential of dendritic cells in type 1 diabetes. *Clin Exp Immunol* 2010;161:197–207
- Phillips B, Nylander K, Harnaha J, et al. A microsphere-based vaccine prevents and reverses new-onset autoimmune diabetes. *Diabetes* 2008;57:1544–1555
- Keselowsky BG, Xia CQ, Clare-Salzler M. Multifunctional dendritic cell-targeting polymeric microparticles: engineering new vaccines for type 1 diabetes. *Hum Vaccin* 2011;7:37–44
- Mahnke K, Qian Y, Knop J, Enk AH. Induction of CD4+/CD25+ regulatory T cells by targeting of antigens to immature dendritic cells. *Blood* 2003;101:4862–4869
- Dhodapkar MV, Steinman RM. Antigen-bearing immature dendritic cells induce peptide-specific CD8(+) regulatory T cells in vivo in humans. *Blood* 2002;100:174–177
- Di Caro V, Phillips B, Engman C, Harnaha J, Trucco M, Giannoukakis N. Retinoic acid-producing, ex-vivo-generated human tolerogenic dendritic cells induce the proliferation of immunosuppressive B lymphocytes. *Clin Exp Immunol* 2013;174:302–317
- Thompson JA, Perry D, Brusko TM. Autologous regulatory T cells for the treatment of type 1 diabetes. *Curr Diab Rep* 2012;12:623–632
- Giannoukakis N, Phillips B, Finegold D, Harnaha J, Trucco M. Phase I (safety) study of autologous tolerogenic dendritic cells in type 1 diabetic patients. *Diabetes Care* 2011;34:2026–2032
- Creusot RJ, Chang P, Healey DG, Tcherepanova IY, Nicolette CA, Fathman CG. A short pulse of IL-4 delivered by DCs electroporated with modified mRNA can both prevent and treat autoimmune diabetes in NOD mice. *Mol Ther* 2010;18:2112–2120
- Huehn J, Polansky JK, Hamann A. Epigenetic control of FOXP3 expression: the key to a stable regulatory T-cell lineage? *Nat Rev Immunol* 2009;9:83–89
- Feili-Hariri M, Falkner DH, Morel PA. Regulatory Th2 response induced following adoptive transfer of dendritic cells in prediabetic NOD mice. *Eur J Immunol* 2002;32:2021–2030
- Creusot RJ, Yaghoubi SS, Kodama K, et al. Tissue-targeted therapy of autoimmune diabetes using dendritic cells transduced to express IL-4 in NOD mice. *Clin Immunol* 2008;127:176–187
- Feili-Hariri M, Falkner DH, Gambotto A, et al. Dendritic cells transduced to express interleukin-4 prevent diabetes in nonobese diabetic mice with advanced insulinitis. *Hum Gene Ther* 2003;14:13–23

40. Palucka K, Banchereau J. Cancer immunotherapy via dendritic cells. *Nat Rev Cancer* 2012;12:265–277
41. Kalantari T, Kamali-Sarvestani E, Ciric B, et al. Generation of immunogenic and tolerogenic clinical-grade dendritic cells. *Immunol Res* 2011;51:153–160
42. Cheever MA, Higano CS: PROVENGE (Sipuleucel-T) in prostate cancer: the first FDA-approved therapeutic cancer vaccine. *Clin Cancer Res* 2011;17:3520–3526
43. Dang Y, Wagner WM, Gad E, Rastetter L, Berger CM, Holt GE, Disis ML. Dendritic cell-activating vaccine adjuvants differ in the ability to elicit antitumor immunity due to an adjuvant-specific induction of immunosuppressive cells. *Clin Cancer Res* 2012;18:3122–3131
44. Bonehill A, Tuyaerts S, Van Nuffel AM, et al. Enhancing the T-cell stimulatory capacity of human dendritic cells by co-electroporation with CD40L, CD70 and constitutively active TLR4 encoding mRNA. *Mol Ther* 2008;16:1170–1180
45. Hilkens CM, Isaacs JD. Tolerogenic dendritic cell therapy for rheumatoid arthritis: where are we now? *Clin Exp Immunol* 2013;172:148–157
46. Whiteside TL, Griffin DL, Stanson J, et al. Shipping of therapeutic somatic cell products. *Cytotherapy* 2011;13:201–213
47. Di Lorenzo TP, Peakman M, Roep BO. Translational mini-review series on type 1 diabetes: systematic analysis of T cell epitopes in autoimmune diabetes. *Clin Exp Immunol* 2007;148:1–16
48. Tree TI, Lawson J, Edwards H, et al. Naturally arising human CD4 T-cells that recognize islet autoantigens and secrete interleukin-10 regulate proinflammatory T-cell responses via linked suppression. *Diabetes* 2010;59:1451–1460
49. Lo J, Peng RH, Barker T, Xia CQ, Clare-Salzler MJ. Peptide-pulsed immature dendritic cells reduce response to beta cell target antigens and protect NOD recipients from type I diabetes. *Ann N Y Acad Sci* 2006;1079:153–156
50. Feili-Hariri M, Dong X, Alber SM, Watkins SC, Salter RD, Morel PA. Immunotherapy of NOD mice with bone marrow-derived dendritic cells. *Diabetes* 1999;48:2300–2308
51. Machen J, Harnaha J, Lakomy R, Styche A, Trucco M, Giannoukakis N. Antisense oligonucleotides down-regulating costimulation confer diabetes-preventive properties to nonobese diabetic mouse dendritic cells. *J Immunol* 2004;173:4331–4341
52. Haase C, Ejmaes M, Juedes AE, Wolfe T, Markholst H, von Herrath MG. Immunomodulatory dendritic cells require autologous serum to circumvent nonspecific immunosuppressive activity in vivo. *Blood* 2005;106:4225–4233
53. Yip L, Su L, Sheng D, et al. Deaf1 isoforms control the expression of genes encoding peripheral tissue antigens in the pancreatic lymph nodes during type 1 diabetes. *Nat Immunol* 2009;10:1026–1033
54. Li J, Park J, Foss D, Goldschneider I. Thymus-homing peripheral dendritic cells constitute two of the three major subsets of dendritic cells in the steady-state thymus. *J Exp Med* 2009;206:607–622
55. Lutz MB. Therapeutic potential of semi-mature dendritic cells for tolerance induction. *Front Immunol* 2012;3:123
56. Xia CQ, Peng R, Beato F, Clare-Salzler MJ. Dexamethasone induces IL-10-producing monocyte-derived dendritic cells with durable immaturity. *Scand J Immunol* 2005;62:45–54
57. Giannoukakis N, Bonham CA, Qian S, et al. Prolongation of cardiac allograft survival using dendritic cells treated with NF- κ B decoy oligodeoxyribonucleotides. *Mol Ther* 2000;1:430–437
58. Ma L, Qian S, Liang X, et al. Prevention of diabetes in NOD mice by administration of dendritic cells deficient in nuclear transcription factor-kappaB activity. *Diabetes* 2003;52:1976–1985
59. Huck SP, Tang SC, Andrew KA, Yang J, Harper JL, Ronchese F. Activation and route of administration both determine the ability of bone marrow-derived dendritic cells to accumulate in secondary lymphoid organs and prime CD8+ T cells against tumors. *Cancer Immunol Immunother* 2008;57:63–71
60. Ruffner MA, Robbins PD. Dendritic cells transduced to express interleukin 4 reduce diabetes onset in both normoglycemic and prediabetic nonobese diabetic mice. *PLoS ONE* 2010;5:e11848
61. Staeva TP, Chatenoud L, Insel R, Atkinson MA. Recent lessons learned from prevention and recent-onset type 1 diabetes immunotherapy trials. *Diabetes* 2013;62:9–17
62. Ziegler AG, Nepom GT. Prediction and pathogenesis in type 1 diabetes. *Immunity* 2010;32:468–478
63. Akirav EM, Lebastchi J, Galvan EM, et al. Detection of β cell death in diabetes using differentially methylated circulating DNA. *Proc Natl Acad Sci USA* 2011;108:19018–19023
64. Erener S, Mojibian M, Fox JK, Denroche HC, Kieffer TJ. Circulating miR-375 as a biomarker of β -cell death and diabetes in mice. *Endocrinology* 2013;154:603–608
65. Ferrannini E, Mari A, Nofrate V, Sosenko JM, Skyler JS; DPT-1 Study Group. Progression to diabetes in relatives of type 1 diabetic patients: mechanisms and mode of onset. *Diabetes* 2010;59:679–685
66. Dhodapkar MV, Steinman RM, Krasovsky J, Munz C, Bhardwaj N. Antigen-specific inhibition of effector T cell function in humans after injection of immature dendritic cells. *J Exp Med* 2001;193:233–238
67. Turley SJ, Lee JW, Dutton-Swain N, Mathis D, Benoist C. Endocrine self and gut non-self intersect in the pancreatic lymph nodes. *Proc Natl Acad Sci USA* 2005;102:17729–17733